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Glycomimetic Inhibitors of Mycobacterial Glycosyltransferases: Targeting the TB Cell Wall

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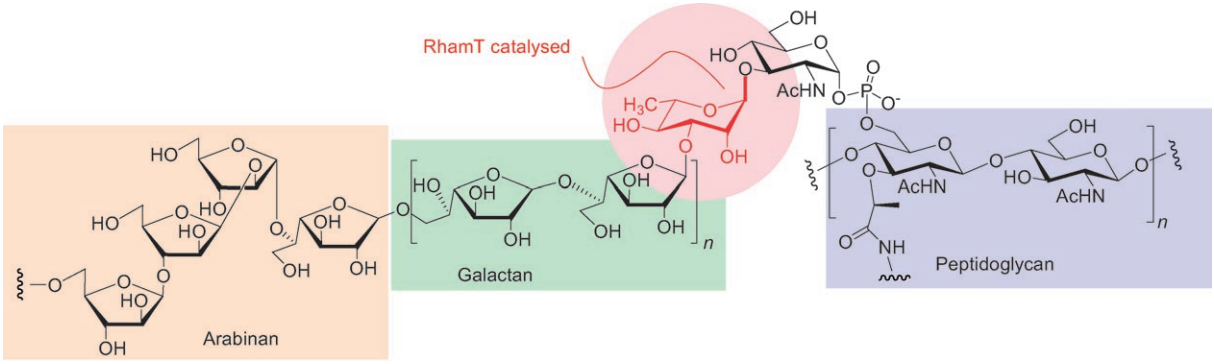
Tuberculosis (TB) is the cause of more than a million deaths a year[1] and is believed to infect a third of the world's population.[2] Emergent multidrug-resistant strains[3] of *Mycobacterium tuberculosis*, the organism that causes the disease, and difficulties in treating immunocompromised individuals have further increased the urgency of the threat.[4] The cell wall of mycobacteria is formed by polysaccharides and lipids[5,6] essential for cell growth and survival in the host,[7] and the importance of its integrity is confirmed by the effectiveness of methods that disrupt cell wall biosynthesis.[8] The major polysaccharide region is joined to peptidoglycan by the so-called bridging region, which contains a critical and unique disaccharide phosphodiester linker (Scheme 1). The presence of an L-rhamnosyl residue in this linker region is a striking drug target opportunity,[9] as it is a sugar that is not found in mammalian cells. A key step in the proposed biosynthetic pathway of the bacterial cell wall[9,10] is the rhamnosyltransferase-mediated (RhamT) glycosylation of GlcNAc-diphosphoprenyl acceptor by the dTDP-Rha donor. Since this class of enzyme is not found in man, RhamT inhibition is an avenue for a potentially nontoxic treatment of TB.[8]

Iminosugars have been widely studied as inhibitors of carbohydrate-processing enzymes such as glycosidases[11,12] and glycosyltransferases (GTs).[13–15] However, inhibition of L-rhamnosyl-processing enzymes has not been widely explored[16,17] and there are no known Rham-T inhibitors. We report here a novel, ready and modular methodology to synthesise iminosugar L-rhamnomimetics that are effective inhibitors of L-rhamnose processing enzymes including importantly the *Mycobacterium* biosynthesis of the bridging disaccharide region.

Two parallel synthetic strategies (Scheme 2) allowed ready access to libraries of both α- and β-pseudoanomers based on the L-rhamno-aza-*C*-glycoside[18] scaffold **1**. From the key divergent intermediate **3**,[16] through diastereoselective reduction or nucleophilic addition coupled with variation of the timing of substituent (**R**) introduction we could control both the pseudoanomeric configuration and identity of **R** in a wide-ranging manner. This has allowed us to map binding interactions and the effect of configuration in Rha-processing enzymes.

Imine intermediates to rhamnomimetics **1αa–h** and **1βa–p** were readily accessed from azidocarbonyl precursors (**5** and **6**, respectively) through either intramolecular Staudinger aza-Wittig ring-closing metathesis or hydrogenolytic reductive cyclisation, respectively. Substituents were introduced at an early (by nucleophilic addition to azidolactone **3**) or late stage (by nucleophilic addition to cyclic polyhydroxyimine **6**).



Scheme 1. *Mycobacterium tuberculosis* cell-wall glycan and "linker" region.

Azidolactone **3** was prepared from D-gulonolactone in six steps.[16] Representative substituents were introduced to the **1β** scaffold through nucleophilic addition including the use of lithiated alkoxymethyl nucleophiles.[19] Few examples of nucleophilic additions to 5-azido 1,4-lactones have been reported;[20] pleasingly all additions (Table 1) selectively yielded corresponding azidolactols (**4b–p**, 68–100%) with no sign of overaddition.[21–28] Hydrogenation gave protected L-rhamnopiperidines

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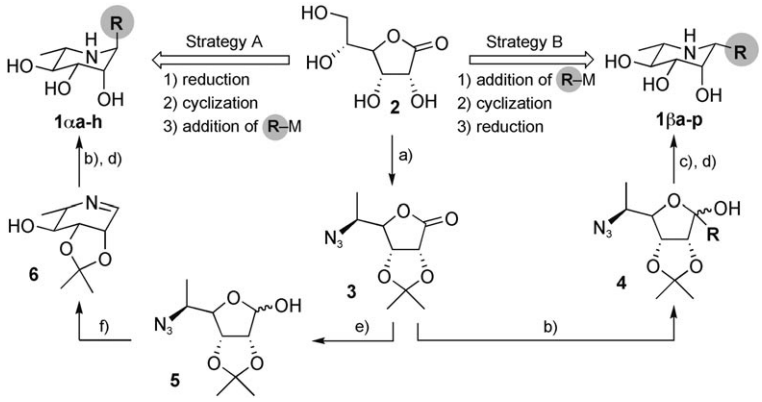
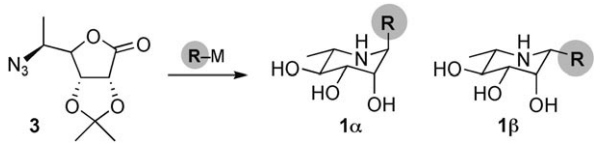


Scheme 2. Synthesis of the α,β- aza-*C*-rhamnomimetics. Reagents and conditions: a) 5 steps: i) 2,2-dimethoxypropane, PTSA, acetone. ii) AcOH-H_2O. iii) CBr_4, PPh_3, THF. iv) H_2, Pd-C, EtOH, NEt_3. v) Tf_2O, py, DCM, −40 °C then NaN_3, DMF. b) **R**M, THF, −78 to 60 °C or −78° to RT; c) H_2, Pd-C, EtOH; d) TFA/H_2O; e) DIBAL-H, THF, −78 °C; f) $Ph_2P(CH_2)_2PPh_2$, THF, 50 °C. See Table 1 for range of **R** and yields. PTSA = *p*-toluenesulfonic acid; Tf = trifluoromethanesulfonyl; DCM = dichloromethane; DMF = dimethylformamide; DIBAL-H = diisobutylaluminium hydride.

Table 1. Organometallic additions: reaction conditions and yields.



	RM	Side chain	Method[a]	Yield[b] [%] 1α	1β
1	iBu_2AlH	**a**	A	100	
2	MeMgCl	**b**	B		91
3	EtMgBr	**c**	B		98
4	EtMgBr	**c**	A	67	
5	VinylMgBr	**d**	B		93
6	VinylMgBr	**d**	A	81	
7	AllylMgBr	**e**	B		72
8	AllylMgBr	**e**	A[d]	75	
9	EthynylMgBr	**f**	A	82	
10	PhMgCl	**g**	B		92
11	PhMgBr	**g**	A[e]	31	
12	2-NaphthylMgBr	**h**	B		99
13	2-NaphthylMgBr	**h**	A[e]	24	
14	*n*BuLi	**i**	B		96
15	OctylMgBr	**j**	B		97
16	MeOPhMgBr	**k**	B		99
17	BnMgBr	**l**	B		82
18	$MeOPhCH_2MgCl$	**m**	B		69
19	BiphenylMgBr	**n**	B		95
20	PhenanthrylMgBr	**o**	B		70
21	$MeOPhCH_2OCH_2Li$[c]	**p**	B		68

[a] Method A: imine formation using $Ph_2P(CH_2)_2PPh_2$ in THF, 50 °C, 15 min prior to addition to Grignard solution. Method B: organometallic (1.2–1.3 equiv) additions in THF −78 °C to −60 °C, 1-2 h then hydrogenation. [b] Isolated yields of **1** from **3** over 3–4 steps; configuration determined by NOESY spectroscopy. [c] RLi prepared in situ with $MeOPhCH_2OCH_2SnBu_3$ and *n*BuLi. [d] Imine formation also with PBu_3 (1.2 equiv), THF, 30 min, rt. [e] Imine formation was with PPh_3 (3.0 equiv), THF, 50 °C, 3 h. See the Supporting Information for full details.

as single diastereoisomers in high yields (82–92%) by reduction of the azido group and subsequent intramolecular reductive amination. Isopropylidene deprotection using TFA followed by ion-exchange gave **1αa**[16] and novel aza-*C*-β-rhamnomimetics **1βb–p** (70–98%; Scheme 2).

The synthesis of the corresponding aza-*C*-α-mimetics was achieved using organometallic additions[29–32] to partially protected cyclic imine **6**, accessed by the Staudinger aza-Wittig reaction.[33] Optimal yields were obtained from addition of imine to Grignards (inverse addition[31]), as representative, model nucleophiles, (see Table 1, Method B) which successfully yielded adducts despite the presence of an unprotected hydroxy group (4-OH) in **6**. Excellent diastereoselectivities were observed in all of the additions (*de* > 98%), consistent with a conformation enforced by 2,3-*O*-isopropylidine that favours nucleophile approach from the resulting convex α face. Subsequent TFA deprotection yielded the aza-*C*-α-rhamnomimetics **1αc–h** (85–95%).

Having readily generated a range of rhamnomimetics **1** as potential enzyme inhibitors, all the compounds were assayed against a variety of representative prokaryotic and eukaryotic glycosidases:[34] all showed some inhibition against α-rhamnosyl processing enzyme naringinase at 1 mM. In particular, the naphthyl derivatives **1αh** and **1βh** were found to be potent and selective competitive inhibitors with $K_i = 1.0$ and 0.26 μM, respectively. Importantly, this inhibition is highly selective: no inhibition was exhibited by other glycosidases capable of binding a range of sugar configurations found in mammalian glycobiology (β-glucosidase, α- and β-mannosidase, α- and β-galactosidase, α-fucosidase). Excitingly, this suggested that the use of such compounds in mammalian systems may be possible without concomitant side-effect inhibition of host enzymes.

Following this exciting confirmation of the configurational mimicry of **1αc–h** and **1βa–n** in Rha-processing systems, we moved to evaluate inhibition[35] of mycobacterial systems.[8,36,37] Interestingly, of all the compounds, unsubstituted **1a** was the most potent (38% at 100 μM). Reasonable inhibition was also exhibited only by those bearing β-alkyl and β-aryl pseudoanomeric substituents (**1βb** 25%, **1βc** 32% **1βi** 19%, **1βj** 26%, **1βm** 23%, and **1βp** 30%), including the naphthyl analogue **1βh** (25%). To the best of our knowledge these are the first rhamnose-like inhibitors of mycobacterial RhamT to date and suggest some early potential for success in targeting this enzyme. This inhibition is configurationally sensitive and selective: no inhibition was displayed by any of the **1α** α-series and none of the compounds inhibited (see the Supporting Information) the RmlC/D enzymes that are dTDP-6-deoxy-4-ketoglucose epimerase/dTDP-Rha synthase, respectively, responsible for the formation of the donor sugar dTDP-β-L-Rha.

The striking lack of activity by any of the α-configured mimetic series **1α** and the inhibition by only members of the β series **1β** towards the mycobacterial system suggests mimicry of the donor substrate dTDP-β-L-Rha, or of a transition state in which a group is found at the β face of a rhamnosyl unit. Indeed, addition of pure, synthetic (for synthesis see the Supporting Information) rhamnosyltransferase substrate dTDP-β-

Rha to the mycobacterial assay system reduced inhibition in a manner consistent with competition with the inhibition of **1a**. This substrate protection/rescue suggested competitive inhibition by **1a** of the donor site of the L-rhamnosyltransferase.

Some of the most active compounds (for example, **1βj** (*n*-octyl), **1βm** (*p*-methoxybenzyl)) bear large substituents at their β face. In contrast, phenyl-(**1βg**)- and *p*-methoxy-phenyl-(**1βk**)-substituted compounds showed little or no inhibitory activity. Together these structure–activity data suggest a potentially deep but narrow-necked hydrophobic pocket at the β face within the inhibition (donor) site.

In conclusion, this paper describes the efficient synthesis of α- and β-homonojirimycin analogues of L-rhamnopyranose. These inhibitors are directly effective against the activity of isolated mycobacterial membrane, with promising but, as yet, unoptimised, inhibitory activity towards a mycobacterial pathway involving rhamnosyltransferase, a validated enzyme target in the fight against tuberculosis.

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[34] These included β-glucosidase (sweet almonds), α-mannosidase (jack beans), β-mannosidase (snail acetone powder), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*), α-fucosidase (bovine kidney), and α-rhamnosidase (*Penicillium decumbens*, naringinase).
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